

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Eijiro WATANABE et al.

Serial No.: 08/992,914

Group: 1638

Filed: December 18, 1997

Examiner: D.H.Kruse

For: RAFFINOSE SYNTHASE GENES AND THEIR USE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

## ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:

MAPP SITKTATLQDVISTIDIGNGNPLFSITLDQSRDFLANGHPFLTQV  
PPNITT TTTTASSFLNLKSNKDTIPNNNNTMLLQQGCFVGFnSTEPKSH  
HVVPLGKLKGIKFMSIFRFKVWWTTTHWVGTVNGQELQHETQMLILDKNDSL  
GRPYVLLLPILENTFRTSLQPGLNDHIGMSVESGSTHVTGSSFKACLYIH  
LSNDPYSILKEAVKVIQTQLGTFKTLEEKTAPSIIIDKFGWCTWDAYLK  
HPKGVWEGVKSLTDGGCPPGFVII DDGWQSICHDDDDDEDSGMNRTSAGE  
QMP CRLVKYEENSKFREYENPENGKKGLGGFVRDLKEEFGSVESVYVWH  
ALCGYWGGVRPGVHGMPKARVVVPKVSQGLKMTMEDLAVDKIVENGVGLV  
PPDFAHEMF DGLHS HLESAGIDGVKVDVIHLLELLSEEYGGRVELARAYY  
KALTSSVKKHFKGNGVIASMEHCNDFFLGTEAISLGRVGDDFWCSDP SG  
DPNGTYWLQGCHMVHCAYNSLWMGNFIQPDWDMFQSTHPCAEFHAA SRAI  
SGGPIYVSDCVGNHNFKLLKSLVLPDGSILRCQHYALPTRDCLFEDPLHN  
GKTM LKIWNLNKYTGVLGLFNCQGGGWCPEARRNKSVSEFSRAVTCYASP  
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKLRLMKCSDRLKVSLEPFSFEL  
MTVSPVKVFSKRFIQFAPIGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC  
GEMSVFASEKPVCCIIDGVKVKFLYEDKMARVQILWPSSSTLSVQFLF

Sc-03:

MAPSF SKENS KTCDEVANHDCNTCP IISLEESNF MVNGHVILSQVPSNI  
TAISKMGFDGLFVG FDAPEPKARHVV SVGQLKGIPFMSIFRFKVWWTTHW  
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD  
YVDICVESGSTKVGDSFRAVLYIRAGPDFPKLIDKTMKEVQAH LGTFKL  
LDDKTPPGIVDKFGWCTWDAYLK VEXYGVWEGVKL VENGVPPGLVLI  
DGWQSICHD DDPITDQEGINRTSAGEQMP CRLIKYEENFKFRDYKSPNIM  
GHEDHPNMG MRAFVRDLKEE FKTV EH VV WHAFTGYWGGVRPNV PGLXEA  
QVVT PKLSPGLEMTMEDLAVDKIVNNNGICLVQPDKAQELYEG LHSHLENC

GIDGVKVDVIHLLEMMAEDYGGRVELAKTYYKAI  
TESVRKHFKGNGVIAS  
MEQCNDFMLLGTEI CLGRVGDDFWPTDPSGDINGTYWLQGCHMVHCAYN  
SLWMGNFIHPDWDMFQSTHPCAEFHAA  
SRAISGGPIYVSDVVGKHNIPLL  
KRLVLADGSILRCEYHALPTKDCLFVDPLHDGKMLKI  
WNLNKYNGVLGV  
FNCQGGGWSRESRKNL  
CFSEYSKPISCKTSPKDVEWENGHKPFP  
IKGVEC  
FAMYFTKEKKLILSQLSDTIEISLDPFDYELIVVSPMT  
ILPWESIAFAPI  
GLVNMLNAGGA  
VKSLDISEDNEDKMVQVG  
IKGAGEMMVSSEKPKACRVN  
GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYL

Sc-04 (truncated) :

MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT  
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR  
FKVWWTTHWVGNSNGHELEHETQMMLLDKNDQLGRPVLILPILQASFRAS  
LQPGLDDYDVCMESGSTRVC  
CGSSFGSCLYVHVGHDPYQLLREATKVVRM  
HLGTFKLLEEK  
TAPVIIDKFGWCTWD  
AFYLVHP  
SGVWE  
GVKGLVEGGCP  
PGMVLIDDGWQAICH  
DEDPTIDQEGMKRTSAGEQ  
MP  
CRLV  
KLEEN  
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VRPK  
VPGMPQ  
AKV  
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NSLWMGNFIQPD  
DWDMFQSTHPCAE  
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Sc-05 :

MAPP  
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PsRFS:

MAPP SITKTATQQDVISTVDIGNSPLLSISLDQSRNFLVN GHPFLTQVPP  
N I TTTTTSTPS PFLDFKS N KDTIANNNTLQQQGCFVG FNTTEAKSHVV  
PLGKLKGIKFTSIFRFKVWWTTTHWVGTNGHELQHETQILILDKNISLGRP  
YVLLLPILENSFRTS LQPGNDYVDMSVESGSTHVTG STFKACLYLHL SN  
DPYRLVKEAVKVIQT KLGTFKTLEEKTPPSIEKFGWCTWD AYLVHPK  
GVWEGVKALTDGGCPPGFVII DDGWQSISHDDDPVTERDGMNRTSAGEQ  
MPCRLIKYEENYKFREYENG DNGGKKGLVG FVRDLKEEFRS VESVYVWA  
LCGYWG GVRPKVCCMPEAKVVVPKLSPGVKMTMEDLA VDKIVENGVL VP  
PNLAQEMFDGIHS HLESAGIDGVKVDV IHLLELLSEYGG RVELAKAYYK  
ALTSSVNKHFKGN GVIASMEHCNDFFLLGTEAISLRVGDDFWCCDP SGD  
PNGTYWLQGCHMVHCAYNSLWMGNFIHPDWDMFQSTHPCAEFHAASRAIS  
GGPVYVSDCVGNHNFKLLKSFPVLPDGSILRCQHYALPTRDCLFEDPLHNG  
KTM LKIWNLNKYAGV LGFNCQGGGWC PETRRNKSASEF SHAVTCYASPE  
DIEWCNGKTPMDIKGVDFAVYFFKEKKLSMKCSRLEVSLEPFSFELM  
TVSPLKVFSKRIIQFAPIGLVNMLNSGGAVQSLEFDD SASLVKIGVRGCG  
ELSVFASEKPVCC KIDGVSV EFDYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05:

MAPSFKN GGSNVV SF DGLNDMSSPFAIDGSDFTVN GHSFLSDV PENIVAS  
PSPYTSIDKSPVSVGCFVGFDASEPDSRH VVISGKLDIRFMSIFRFK V W  
WTTHWVGRNGGDLESETQIVILEKSDSGRPYVFLPIVEGPFR TSIQPGD  
DDFVDVCV ESGSSK VVDASFRSMLYLHAGDDPFALVKEAMKIVRTHLGTF  
RLLEEKTP PGIVDKFGWCTWD AYLTVHPQGVIEGVRHLVDGGC PPLV L  
IDDGWQSIGH DSDPITKEGMN QTVAGEQMP CRLLK FQEN YKFRDYVNPKA  
TGPRAGQKGMKAF IDELKGEFKTVEHVYVWHALCGYWGLRPQVPG LPEA  
RVIQPVLS PGLQMTMEDLA VDKIVLHKVGLVPPEKAEE MY EGL HAH LEKV  
GIDGVKIDV IHLLEM CEDY GGRV DLAKAYYKAMTKSINKHFKGNGVIAS  
MEHCNDMF MFLGTEAISLRVGDDFWCTDP SGDPNGTFWLQGCHMVHCAND  
SLWMGNFIHPDWDMFQSTHP CAAF HAASRAISGGPIYVSDSVGKHNF DLL  
KKLVLPDGSILRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVIGA  
FNCQGGGWC RETRNQCF SQYSKRVTSKTNPKDIEWHSGENPISIEGVKT  
FALYLYQAKKLILSKPSQDLDIA LD PFEFELITVSPVTKLI QTSLHF API  
GLVNMLNTSGAIQSVDYDDDLSSVEIGVKGC GEMRVFASKKPRACRIDGE  
DVGFKYDQDQM VVVQVWPWIDSSSGGISVIEYL F

HvSIP:

MTVTPQITVGDGRLAVRGRTVLSGVPDNVTA AHAAGAGLVDGAFVGATAA  
EAKSHHVFTFGTLRDCRFMCLFRFKLWWMTQRMGTS GRDVPLETQFILIE

VPAAAGNDDGDSSDGDSEPVYLVMLPLEGQFRVLQGNDQDELQICIES  
GDKAVETEQGMNNVVHAGTNPFDTITQAVKAVEKHTQTFHHREKKTVPS  
FVDWFGWCTWDASFYTDVTADGVKQGLRSAEGGAPPRFLIIDDGWQQIGS  
ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDETAKK  
EHGVKSYYVWHAMAGYWGGVKPSAAGMEHEYEPALAYPVQSPGVTGNQPDI  
VMDSLSVGLGLVHPRRVHRFYDELHAYLAACGVGVKVDVQNI VETLGA  
GHGGRVALTRAYHRALEASVARNFPDNGCISCMCHNTDMLYSAKQTAVVR  
ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFHSLHPAAEYHGA  
ARAIGGCPYVSDKPGNHNFLLRKLVLPDGSVLRALPGRPTRDCLFSD  
PARDGASLLKIWNMMNKCAGVVGVFNCQGAGWCRVAKKTRIHDEAPGTLTG  
SVRAEDVEIAQAAGTGDWGGEAVVYAHRAGELVRLPRGATLPVTLKRLE  
YELFHVCVPRAVAPGVSFAPIGLLHMFNAGGAAVEECTVETGEDGNAVGL  
RVRCGRFGAYCSRPAKCSVDSADVEFTYDSDTGLVTADVPVPEKEMYR  
CALEIRV

AmSTS:

MAPPYDPIPIPMMSAILNFLSSTVKDNSFELLGTLTSVKNVPILTDIPS  
NVSFSFSSIVQSSEAPVPLFQRAQSLSSSGGFLGFSQNEPSSRLMNSLG  
KFTDRDFVSIFRFKTWWSTQWVGTGSDIQMETQWIMLDVPEIKSYAVVV  
PIVEGKFRSALFPGKDGHILIGAESGSTKVKTNSFDAIAYVHSNPyTL  
MRDAYTAVRVHLNTFKLIEEKSAAPLVNKFGWWTWDAFYLTVEPAGIYHG  
VQEFAADGLTPRFLIIDDGWQSINNDDNDPNEDAKNLVLGGTQMTARLHR  
LDECEKFRKYKGGSMSGPNRPPFDPKPKLISKAIIEVAEKARDKAAQ  
SGVTDLARYEAEIEKLTKEQDQMFGGGEETSSGKSCSSCSCKSDNFGMK  
AFTKDLRTNFKGDDIYVWHALAGAWGGVRPGATHNAKIVPTNLSPGLD  
GTM TD LAVVKIEGSTGLVDPDQAEDFYDSMHSYLSSVGITGVKVDVIHT  
LEYI SEDYGGRVELAKAYYKGLSKSLAKNFNGTGLISSMQQCNDFFLLGT  
EQISMGRVGDDFWFQDPNGDPMGVYWLQGVHMIHCAYNSWMQF IQPDW  
DMFQSDHPGGYFHAGSRAICGGPVYVSDLGGHNFLLKKLVFNDGTIPK  
CIHFALPTRDCLFKNPLFDSKTIK IWNFNKYGGVIGAFNCQGAGWDPKE  
QRIGYSQCYKPLSGSVHVSGIEFDQKKEASEMGEAEYAVLSEAELKS  
LATRDSDPIKITIQSSTFEIFS FVP I KKLGEGVKFAPIGLTNLFNAGGTI  
QGLVYNEGIAKIEVKGDGKFLAYSSVVPKKAYVNGAEKVFAWSGNGKLEL  
DITWYEECGGISNVTFVY

PsSTS-1:

MAPPLNSTTSNLIKTESIFDL SERKFKVKGPLFHDVPEVNSFRSFSSIC  
KPSESNAPPSSLQKVLAYSHKGFFGFSHETPSDRLMNSIGSFNGKDFLS  
IFRFKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIIPPIIEKCFRS  
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYSAIR  
VHLNSFRLLEEKTIIPNLVDKFGWCTWDASFYLTVNPIGIFHGLDDFSKGKV

EPRFVIIDDGWQSISFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK  
YESGLLGPNSPPYDPNNFTDLILKGIEHEKLRKKREEAISSKSSDLAEI  
ESKIKKVVKEIDDLFGGEQFSSGEKSEMKS EYGLKAFTKDLRTKFGLDD  
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS  
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGRVDA  
KVYYEGLTKSIVKNFNGNGMIA SMQHCNDFFLGTKQISMGRVGDDFWFQ  
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVCASFHAG  
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN  
PLFDHTTVLK IWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKP IPG  
TVHVTVEWDQKEETSHLGKAEEYVVYLNQAEELSLMTLKSEPIQFTIQP  
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK  
GGGSFLAYSSES PKKFQLNGCEVDFEWLGDGKLCVNVPWIEACGVSDME  
IFF

PsSTS-2:

MAPPLNSTTSNLIKTESIFDL SERKFVKVKGPLFHDVPENVSFRSFSSIC  
KPSESNAPPSSLQKV LAYSHKGFFGFSHETPSDR LMNSLGSFNGKDFLS  
IFRFKTWWSTQWI GKSGSDLQM ETQWILIEVPETKSYVVIIP IIEKCFRS  
ALFPGFNDHVKI IAEGSTKVKESTFNSIAYVHFSEN PYDLMKEAYIAIR  
VHLNSFRLLEEKTI PNLDKFGWCTWD AFYLTVNPIGIFHGLDDFSKGGV  
EPRFVIIDDGWQSISFDGC DPNEDAKNLVLGGEQMSGRLHRFDECYKFRK  
YESGLLGPNSPPYDPKKFTDLILKGIEHEKLRKKREEAISSKSSDLAEI  
ESKIKKVVKEIDDLFGGEQFSSVEKSEMKS EYGLKAFTKDLRTKFGLDD  
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS  
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGRVDA  
KVYYEGLTKSIVKNFNGNGMIA SMQQCNDFFLGTKQISMGRVGDDFWFQ  
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVCASFHAG  
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN  
PLFDHTLLK IWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKP IPG  
TVHVTQVEWDQKEETSHFGKAEEYVVYLNQAEELCLMTLKSEPIQFTIQP  
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK  
GGGSFLAYSSES PKKFQLNGCEVDFEWLGDGKLCVNVPWIEACGVSDME

SaSTS:

MAPPNDPISSIFSPLISVKKDNAFELVCGKLSVKNVPLLSEIPSNTFKS  
FSSICQSSGAPAPLYNRAQSLSNCGGFLGFSQKESADSVTNSLGKFTNRE  
FVSIFRFKTWWSTQWVGTSGSDIQMETQWIMLNPEIKSYAVVIPIVEGK  
FRSALFPGKDGHVLISAEGSTCVKTTSFTSIAVHVSDNPYTLMDGYT  
AVRVHLDTFKLIEEKSAAPPLVNKFGWCTWD AFYLTVEPAGIWNGVKEFSD  
GGFS PRFLIIDDGWQSINIDGQDPNEDAKNLVLGGTQMTARLHRFDEC EK  
FRKYKGGSMMPKVPYFDPKKPKLLISKAIEIEGVEKARDKAIQSGITDL

SQYEIKLKKLNKELDEMFGGGNDEKSSKGCSDCSCKSQNSGMKAFTND  
LRTNFGLDDIYVWHALAGAWGGVKPGATHLNKIEPCKLSPGLDGTMD  
LAVVKİLEGSİGLVHPDQAEDFYDSMHYSLSKVGİTGVKVDIHTLEYVS  
ENYGGRVELGKAYYKGLSKSLKKNFNGSGLİSSMQQCNDFFLLGTEQISM  
GRVGDDFWFQDPNGDPMGVFWLQGVHMIHCAYNSWMGQIIHPDWDMFQS  
DHCSAKFHAGSRAICGGPVYVSDSLGGHDFDLLKKLVFNDGTIPKCİHFA  
LPTRDCLFKNPLFDSTKILKİWNFNKYGGVVGAFNCQGAGWDPKEQRIKG  
YSECYKPLSGSVHSDİEWWDQKVEATKMGEAEYAYVLTESEKLLTTPE  
SDPİPFTLKSTTFEİFSFVPİKKLGQGVKFAPİGLTNLFNSGGTİQGVVY  
DEGVAKIEVKGDGKFLAYSSVPKRSYLNGEEVEYKWSGNKGVEVDVPWY  
EECGGISNITFVF

VaSTS :

MAPPNDPVNATLGLEPSEKVFDSLGDKLTVGVVLLSHVPENVTSSSS  
İCVPRDAPSSİLQRVTAASHKGFLGFSHVSPSDRLİNSLGSRGRNFLS  
İFRFKTWWSTQWVGNSGSDLQMETQWİLİEVPETESYVVİİPİİEKSFRS  
ALHPGSDDHVKİCAESGSTQVRASSFGAİAYVHVAETPYNLREAYSALR  
VHLDŞFRLLEEKTVPİRİVDKFGWCTWDAYLTVNPGVWHLKDFSEGKV  
APRFVVİDDGWQSVNFDDEDPNEDAKNLVLGGEQMTARLHRFEEGDKFRK  
YQKGLLLGPNAŞFPETİKELİSKGİEAELGKQAAAİSAGGSDLAEİE  
LMİVKVREEİDDLFGGKGKESNESGGCCCAAECGGMKDFTTDLRTEFKG  
LDDVYVWHALCGGWGGVRPGTTHLDISKİİPCKLSPGLVGTMKDLAVDKIV  
EGSİGLVHPHQANDLYDSMHSYLAQTGVTGVKİDVİHSLEYVCEYGGRV  
EİAKAYYDGLTNSİİKNFNGSGİİASMQQCNDFFFLGTKQİİPFGRVGDDF  
WFQDPNGDPMGVFWLQGVHMIHCSYNSLWMGQIIQPDWDMFQSDHECAKF  
HAGSRAICGGPVYVSDSVGSHDFDLİKKLVFPDGTVPKCIYFPLPTRDCL  
FRNPLFDQKTVLKİİWNFNKYGGVİGAFNCQGAGWDPKGKKFKGFPECYKA  
İSCTVHVTEVEWDQKKEAHMGKAEEYVVYLNQAEVLHLMTPVSEPLQLT  
İQPSTFELYNFVPVEKLGSNSIKFAPİGLTNMFNSGGTİQELEYIEKDVK  
VKVKGGGRFLAYSTQSPKKFQLNGSDAAFQWLPGKLTLNLAWIEENDGV  
SDLAIFF

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenetic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenetic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. In the molecular phylogenetic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

Sc-04 (full-length) :

MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT  
PSPIDAKSSKNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR  
FKVWWTTHWVGSGNGHELEHETQMMLLDKNDQLGRPFVILPILQASFRAS  
LQPGLDDYVDVCMESGSTRVCGSSFGSCLYHVHGHDYPYQLLREATKVVRM  
HLGTFKLLEEKTAAPVIIIDKFGWCTWDASFYLKVHPSGVWEGVKGLVEGGCP  
PGMVLIDDGWQAICHDEDPITDQEGMKRTSAGEQMPCRLVKLEENYKFRQ  
YCSGKDSEKGGMGAFVRDLKEQFRSVEQYYVWHALCGYWGGVRPKVPGMPQ  
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLYEGLHSRLES  
AGIDGVKVDVIHLLEMSEYYGGRVELAKAYYKALTASVKKHFKGNGVIA  
SMEHCNDFFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY  
NSLWMGNFIQPDWDMFQSTHPCAEFHAAASRAISGGPVYVSDCVGKHNFKL  
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKMLKIWNLNKYTGVLG

LFNCQGGGWCPVTRRNKSASEFSQTVTCLASPQDIEWNGKSPICIKGMN  
VFAVYLFKDHLKLMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP  
IGLVNMLNTGGAIQSMEFDNHIDVVVK1GVRCGEMKVFASEKPVVSCKLDG  
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

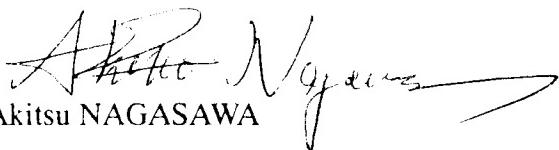
As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x\_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6<sup>th</sup> day of September, 2005



Akitsu NAGASAWA

Table 1

Code	Protein*	Organism	Accession**	Reference	Author/Assignee
Sc-03	RFS	<i>Beta vulgaris</i>	E37133	09/301,766	Sumitomo Chemical
Sc-05	RFS	<i>Brassica juncea</i>	E36417	09/301,766	Sumitomo Chemical
Sc-02	RFS	<i>Vicia faba</i>	E24423	08/992,914	Sumitomo Chemical
Sc-04	RFS	<i>Glycine max</i>	E24424	08/992,914	Sumitomo Chemical
Aj-05	RFS	<i>Cucumis sativus</i>	AF073744	Family GH36***	Ohsumi et al.
PsRFS	RFS	<i>Pisum sativum</i>	AJ426475	Family GH36	Peterbauer et al.
HvSIP	SIP	<i>Hordeum vulgare</i>	M77475	Family GH36	Heck et al.
PsSTS-1	STS	<i>Pisum sativum</i>	AJ311087	Family GH36	Peterbauer et al.
PsSTS-2	STS	<i>Pisum sativum</i>	AJ512932	Family GH36	Peterbauer et al.
VaSTS	STS	<i>Vigna angularis</i>	Y19024	Family GH36	Peterbauer et al.
AmSTS	STS	<i>Alonsoa meridionalis</i>	AJ487030	Family GH36	Voitsekhovskaja
SsSTS	STS	<i>Stachys affinis</i>	AJ344091	Family GH36	Pesch and Schmitz

\* Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

\*\* Accession: GenBank Accession Number.

\*\*\* Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: [http://afmb.cnrs-mrs.fr/CAZY/GH\\_36.html](http://afmb.cnrs-mrs.fr/CAZY/GH_36.html))

Table 2

Table 3

Table 4

Fig. 1

[GENETYX : Evolutionary tree]

Date : 2004.2.4

Method: UPGMA

